DP Barcode : D182928
PC Code No : 069105
EEB Out : 8/20/93

To: Brigid Lowery
Product Manager
Special Review and Reregistration Division (H7508W)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 069105
Chemical Name : Alkyl dimethyl benzyl ammonium chloride
Type Product : Microbiocide, sanitizer
Product Name : ADBAC
Company Name : ADBAC Quat Joint Venture
Purpose : review of Estuarine studies

Action Code : 627 Date Due : 09/15/93
Reviewer : Harry A. Winnik Date In EEB: 09/29/92

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)			72-2(A)			72-7(A)		
71-1(B)			72-2(B)			72-7(B)		
71-2(A)			72-3(A)	42479502	Y	122-1(A)		
71-2(B)			72-3(B)	42479503	N S	122-1(B)		
71-3			72-3(C)	42479501	Y	122-2		
71-4(A)			72-3(D)			123-1(A)		
71-4(B)			72-3(E)			123-1(B)		
71-5(A)			72-3(F)			123-2		
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)			124-2		
72-1(B)			72-5			141-1		
72-1(C)			72-6			141-2		
72-1(D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)
N=Unacceptable (Study was rejected)/Nonconcur

RE-EVALUATED
NOV 1, 1995
SEE ATTACHED

DP BARCODE: D182928

REREG CASE #

CASE: 819070
SUBMISSION: S425945

DATA PACKAGE RECORD
BEAN SHEET

DATE: 04/16/93

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REREGISTRATION ACTION: 627 GENERIC DATA SUBMISSION
CHEMICALS: 069105 Alkyl* dimethyl benzyl ammonium chloride *(50%C14, 100.00 %

ID#: 069105
COMPANY:
PRODUCT MANAGER: 72 LARRY SCHNAUBELT 703-308-8058 ROOM: CS1 3C3
PM TEAM REVIEWER: BRIGID LOWERY 703-308-8053 ROOM: CS1 3G6
RECEIVED DATE: 09/15/92 DUE OUT DATE: 09/15/93

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 182928 EXPEDITE: N DATE SENT: 09/24/92 DATE RET.: / /
CHEMICAL: 069105 Alkyl* dimethyl benzyl ammonium chloride *(50%C14, 40%C12,
DP TYPE: 001 Submission Related Data Package
ADMIN DUE DATE: 03/23/93 CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT
DIV : EFED	09/29/92	/ /
BRAN: EEB	/ /	/ /
SECT:	/ /	/ /
REVR :	/ /	/ /
CONTR:	/ /	/ /

* * * DATA REVIEW INSTRUCTIONS * * *

Please review the three studies submitted for reregistration of ADBAC:

72-3a - 42479502
72-3b - 42479503
72-3c - 42479501

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL

2

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

August 11, 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Estuarine Acute Toxicity Studies Submitted to Support Reregistration of Alkyl dimethyl benzyl ammonium chloride (ADBAC), Shaughnessy #069105.

FROM: Anthony F. Maciorowski, Chief 8/19/93
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

TO: Brigid Lowery
Reregistration Branch
Special Review and Reregistration Division(H7508W)

EEB has completed review of three Estuarine Acute Toxicity Studies submitted by ADBAC Quat Joint Venture to support the reregistration of Alkyl dimethyl benzyl ammonium chloride (ADBAC), Shaughnessy #069105. (copies are attached). The following are brief summaries of the reviews:

CITATION: Sved, D.W., J.P. Swigert, and G.J. Smith. 1992. A 96-Hour Static-Renewal Acute Toxicity Test with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Saltwater Mysid (*Mysidopsis bahia*). Project No. 350A-101A. Prepared by Wildlife International Ltd., Easton, MD. Submitted by ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association, Washington, D.C. EPA MRID No. 424795-01.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for an acute estuarine shrimp toxicity study (this study is classified as "core"). The 96-hour LC_{50} value was 0.092 ppm mean measured concentration. Therefore, ADBAC QUAT is classified as very highly toxic to mysids. The NOEC was 0.047 ppm.

RECOMMENDATIONS: N/A.

CITATION: Sved, D.W., J.P. Swigert, and G.J. Smith. 1992. A 96-Hour Static-Renewal Acute Toxicity Test with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Sheepshead Minnow (*Cyprinodon variegatus*). Project No. 350A-102. Prepared by

Wildlife International Ltd., Easton, MD. Submitted by ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association, Washington, D.C. EPA MRID No. 424795-02.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for an acute estuarine fish toxicity study (this study is classified as "core"). The 96-hour LC_{50} value was 0.86 ppm mean measured concentration. Therefore, ADBAC QUAT is classified as highly toxic to sheepshead minnows. The NOEC was 0.68 ppm.

RECOMMENDATIONS: N/A.

CITATION: Sved, D.W., J.P. Swigert, and G.J. Smith. 1992. A 48-Hour Static Acute Toxicity Test with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in Embryo Larvae of the Eastern Oyster (*Crassostrea virginica*). Project No. 350A-103. Prepared by Wildlife International Ltd., Easton, MD. Submitted by ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association, Washington, D.C. EPA MRID No. 424795-03.

CONCLUSIONS: This study is not scientifically sound (this study is classified as "invalid"). Control mortality (47.8%) was unacceptably high. Based on normalized embryo-larvae mortality, the 48-hour LC_{50} was 55.0 ppb mean measured concentration. Based on abnormal development, the 48-hour EC_{50} was 49.1 ppb mean measured concentration. Therefore, ADBAC QUAT is classified as very highly toxic to eastern oysters. The NOEC was 25.0 ppb.

RECOMMENDATIONS: N/A

If you have any questions regarding this submission please contact Harry Winnik, Biologist, 305-7089.

W6RD
SRA
ATTACHED
11-1-95

4

DATA EVALUATION RECORD

1. **CHEMICAL:** Alkyl Dimethyl Benzyl Ammonium Chloride Shaughnessey No. 069105.

2. **TEST MATERIAL:** 1) ADBAC QUAT 80%; Lot No. 7293K, CP-161-1, 010 0879; 80.8% active ingredient; a yellow clear liquid. 2) ADBAC (^{14}C); Lot No. 920326; 12.62 µCi/ml; 97.99-98.74% radiochemical purity; a clear colorless liquid.

3. **STUDY TYPE:** 72-3. Estuarine Shrimp Static-Renewal Acute Toxicity Test. Species Tested: Mysid (*Mysidopsis bahia*).

4. **CITATION:** Sved, D.W., J.P. Swigert, and G.J. Smith. 1992. A 96-Hour Static-Renewal Acute Toxicity Test with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Saltwater Mysid (*Mysidopsis bahia*). Project No. 350A-101A. Prepared by Wildlife International Ltd., Easton, MD. Submitted by ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association, Washington, D.C. EPA MRID No. 424795-01.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 10/21/92

Mary O. Date
8-10-93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 10/21/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: H T Craven
Date: 8/12/93

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute estuarine shrimp toxicity study. The 96-hour LC_{50} value was 0.092 ppm mean measured concentration. Therefore, ADBAC QUAT is classified as very highly toxic to mysids. The NOEC was 0.047 ppm.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile mysids (<24 hours old) were obtained from in-house cultures. Brooding adults were held at the same temperature and in the same dilution water as those used during the test for at least 14 days before juveniles were collected for testing.

B. **Test System:** The test compartments were 500-ml glass beakers with screen-covered holes. The compartments were suspended in 2-l glass beakers filled to a depth of approximately 8 cm. The beakers were indiscriminately positioned in a temperature-controlled water bath set to maintain 25 ±1°C.

The laboratory environment was maintained on a 16-hour light photoperiod with 30-minute dawn and dusk simulations. The light intensity during the test was approximately 240-380 lux.

A dispensing stock solution was prepared using an aliquot of the ^{14}C-ADBAC solution and nonradiolabeled test material diluted to a concentration of 0.30 mg/ml with reverse-osmosis water. The calculated specific activity of the stock was 0.55 mCi/mmol. Test solutions were prepared daily. An appropriate volume of the dispensing stock was added to 2 l of aerated dilution water and stirred for 2 minutes before being dispensed to the test aquaria. Test concentrations were adjusted for the percent purity of the nonradiolabled test material. The aquaria were conditioned with test solution for 23 hours prior to test initiation.

Natural seawater, collected at Indian River Inlet, DE, was diluted with well water, aerated, and filtered before use as test dilution water. The salinity of the dilution water was 25 parts per thousand (ppt).

C. **Dosage:** Ninety-six-hour static-renewal test. Based on the results of a rangefinding test, six nominal concentrations (0.029, 0.048, 0.080, 0.13, 0.22, and 0.37 ppm) and a dilution water control were used.

D. **Design:** Mysid shrimp were impartially removed from holding tanks using wide-bore, disposable, polyethylene pipettes and distributed to 25-ml plastic containers until each cup contained 10 individuals. The cups were

indiscriminately assigned and dipped into the test chambers to release the mysids. Two replicates were used, for a total of 20 individuals per concentration. The mysids were fed live brine shrimp nauplii daily during the test.

The test solutions were renewed daily by replacement of 90% of the old solution with freshly-prepared solution.

Observations of mortality and treatment-related effects were made at 4, 24, 48, 72, and 96 hours. The dissolved oxygen concentration (DO) and pH were measured in alternate replicates at the beginning of the test and at each 24-hour observation. The temperature in one of the control chambers was monitored continuously and measured in each replicate vessel once daily.

Water samples from each replicate containing live mysids were collected daily. The concentration of ADBAC QUAT (as mg ADBAC QUAT equivalents/l or ppm) was determined using liquid scintillation counting.

E. **Statistics:** The 96-hour median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) were calculated using nonlinear interpolation with binomial probability.

12. **REPORTED RESULTS:** The mean measured concentrations were 0.030, 0.047, 0.081, 0.13, 0.22, and 0.35 ppm (Table 1, attached).

No mortality or sublethal effects were noted in the control and the lowest two concentrations. The 96-hour LC_{50} was 0.092 ppm with a 95% confidence interval of 0.081-0.13 ppm (Table 2, attached). The no-observed-effect concentration was 0.047 ppm.

During the test, the DO was >60% of saturation (6.1-6.8 mg/l). The pH values ranged from 7.9 to 8.0. The temperature was 24.5-25.5°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Other than those presented above, the authors made no conclusions.

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

Characterization of the test substance was the responsibility of the sponsor. The dates and types of quality assurance audits were reported.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were generally in accordance with the SEP, except for the following:

The recommended temperature for mysid shrimp toxicity tests is 22 ±1°C. The temperature used in the study was approximately 25°C.

The salinity of the dilution water in the study was 25 ppt. The recommended salinity for estuarine shrimp is 10-17 ppt.

The time between solution preparation and test initiation (or renewal) was not reported. Test solutions should be used within 30 minutes of preparation.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the LC_{50} value and obtained the same results (see attached printout).

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for an acute estuarine shrimp toxicity study. The 96-hour LC_{50} value was 0.092 ppm mean measured concentration. Therefore ADBAC QUAT is classified as very highly toxic to mysids. The NOEC was 0.047 ppm.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 10-09-92.

Page ___ is not included in this copy.

Pages 9 through 11 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

RIFICI ADBAC QUAT MYSIDOPSIS BAHIA 10-09-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.35	20	20	100	9.536742E-05
.22	20	20	100	9.536742E-05
.13	20	20	100	9.536742E-05
.081	20	5	25	2.069473
.047	20	0	0	9.536742E-05
.03	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .081 AND .13 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 9.199321E-02

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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DATABASE ENTRY FORM
FOR ACUTE OR CHRONIC TOXICITY STUDIES

1. **Chemical** Alkyl Dimethyl Benzyl Ammonium chloride **Shaughnessy** 069105
2. **Common Name Of Organism Tested** Mysid
3. **Scientific Name** Mysidopsis bahia
4. **Age Of Organisms** < 24 hours
5. **Guideline No.** 72-3.
6. **Type Of Dosing Method (Circle One) Or Study –**

   1. Oral 2. Dietary 3. Reproduction 4. Static
   (5.) Static Renewal 6. Flowthrough 7. Acute Contact
   8. Other _____

7. **% AI Of Test Substance** non Radiolabeled 80.8
8. **Study Duration (Hrs Or Days)** 96h
9. **Dose Type (Circle One)** A. LD50 (B.) LC50 C. EC50 D. MATC
10. **Toxicity Level** A. mg/kg (B.) ppm C. mg/l D. µg/l E. ng/l F. µg/bee G. Other
11. **95% C.L.s** 0.081 - 0.13 LC_{50} = 0.092 binomial prob.
12. **Curve Slope** N/A
13. **NOEL** 0.047
14. **Study Date (YEAR)** 1992
15. **Study Review Date (YEAR)** 1992
16. **Category (Circle One)** (CORE) SUPPLEMENTAL INVALID
17. **MRID of Accession Number** 424795-01
18. **Laboratory** Wildlife Int. Ltd
19. **Reviewer** L. Rifici
20. **For Reproductive Studies (avian or aquatic) Indicate Which Parameter Affected At What Toxicity Level.**

    Eggs Layed _____ % Cracked _____ % Viable _____
    % Live Embryos _____ % Egghatch 14D Survivors _____
    Growth Effected at _____ Other Effects _____

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DATA EVALUATION RECORD

1. **CHEMICAL:** Alkyl Dimethyl Benzyl Ammonium Chloride Shaughnessey No. 069105.

2. **TEST MATERIAL:** 1) ADBAC QUAT 80%; Lot No. 7293K, CP-161-1, 010 0879; 80.8% active ingredient; a yellow clear liquid. 2) ADBAC (^{14}C); Lot No. 920326; 12.62 µCi/ml; 97.99-98.74% radiochemical purity; a clear colorless liquid.

3. **STUDY TYPE:** 72-3. Estuarine Fish Static-Renewal Acute Toxicity Test. Species Tested: Sheepshead Minnow (*Cyprinodon variegatus*).

4. **CITATION:** Sved, D.W., J.P. Swigert, and G.J. Smith. 1992. A 96-Hour Static-Renewal Acute Toxicity Test with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Sheepshead Minnow (*Cyprinodon variegatus*). Project No. 350A-102. Prepared by Wildlife International Ltd., Easton, MD. Submitted by ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association, Washington, D.C. EPA MRID No. 424795-02.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M. Rifici
Date: 10/21/92

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 10/21/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: H. T. Craven
Date: 8/12/93

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute estuarine fish toxicity study. The 96-hour LC_{50} value was 0.86 ppm mean measured concentration. Therefore, ADBAC QUAT is classified as highly toxic to sheepshead minnows. The NOEC was 0.68 ppm.

8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Juvenile sheepshead minnows (*Cyprinodon variegatus*) were obtained from in-house cultures maintained at the same temperature and in the same dilution water as those used during the test. The fish were acclimated to the test conditions for approximately 53 hours before the test. During this period, the fish were not fed and no signs of disease or stress were noted.

All fish used in the test were from the same year class. The average length of 10 control organisms at the end of the test was 20 mm (16-26 mm) with an average wet weight of 0.21 g (0.11-0.37 g).

B. Test System: The test chambers were Teflon-lined 25-l polyethylene aquaria filled with 15 l of test solution. The test solution depth was approximately 17.2 cm. The chambers were indiscriminately positioned in a temperature-controlled environmental chamber set to maintain 22 ±1°C. Lighting in the chamber was maintained on a 16-hour light photoperiod with 30-minute dawn and dusk simulations. The light intensity during the test was approximately 250-1000 lux.

A dispensing stock solution was prepared using an aliquot of the ^{14}C-ADBAC solution and nonradiolabeled test material diluted to a concentration of 0.090 g/ml with reverse-osmosis water. The calculated specific activity of the stock was 0.0088 mCi/mmol. Test solutions were prepared daily. An appropriate volume of the dispensing stock was added to 30 l of aerated dilution water and stirred for 2 minutes before being dispensed to the test aquaria. Test concentrations were adjusted for the percent purity of the nonradiolabeled test material. The aquaria were conditioned with test solution for 23 hours prior to test initiation.

Natural seawater, collected at Indian River Inlet, DE, was diluted with well water, aerated, and filtered before use as test dilution water. The salinity of the water was 25 ppt.

#15

C. **Dosage:** Ninety-six-hour static test. Based on the results of a rangefinding test, five nominal concentrations (0.39, 0.65, 1.1, 1.8, and 3.0 ppm) and a dilution water control were used.

D. **Design:** Sheepshead minnows were impartially removed in groups of two from holding tanks and distributed to the test chambers until each contained 10 fish. Two replicates were used for each level. The instantaneous loading was 0.14 g/l.

The test solutions were renewed daily by replacement of 90% of the old solution with freshly-prepared solution.

Observations of mortality and treatment-related effects were made at 2.5, 24, 48, 72, and 96 hours. The dissolved oxygen and pH were measured in alternate replicates at the beginning of the test and at each 24-hour observation. The temperature in one of the control chambers was monitored continuously. Except on the second day, the temperature of each replicate vessel was measured daily. The salinity of the dilution water was determined at test initiation.

Water samples from each replicate containing live fish were collected daily. The concentration of ADBAC (as mg ADBAC equivalents/l or ppm) was determined using liquid scintillation counting.

E. **Statistics:** The 96-hour median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) were calculated using nonlinear interpolation with binomial probability.

12. **REPORTED RESULTS:** The mean measured concentrations were 0.42, 0.68, 1.1, 1.8, and 2.8 ppm (Table 1, attached).

No mortality or sublethal effects were noted in the control and the lowest two concentrations. The 96-hour LC_{50} was 0.86 ppm with a 95% confidence interval of 0.68-1.1 ppm (Table 2, attached). The no-observed-effect concentration was 0.68 ppm.

During the test, the DO was >60% of saturation (6.2-8.3 mg/l). The pH values ranged from 7.9 to 8.1. The temperature was 21.0-22.2°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Other than those stated above, the authors made no conclusions.

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Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards set forth in 40 CFR Part 160. Characterization of the test substance was the responsibility of the sponsor. The dates and types of quality assurance audits were reported.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with the SEP, except for the following:

The test vessels were Teflon-lined polyethylene aquaria. Stainless steel or glass test vessels are recommended. In this case, the Teflon liner is probably a better substitute.

The salinity of the dilution water in the study was 25.0 ppt with a pH of 8.3. The recommended salinity and pH for sheepshead minnows are 10-17 ppt and 7.7-8.0 or 30-34 ppt and 8.0-8.3.

The time between solution preparation and test initiation (or renewal) was not reported. Test solutions should be used within 30 minutes of preparation.

B. Statistical Analysis: The reviewer used EPA's Toxanal program to calculate the LC_{50} value and obtained the same results (see attached printout).

C. Discussion/Results: This study is scientifically sound and meets the guideline requirements for an acute estuarine fish toxicity study. The 96-hour LC_{50} value was 0.86 ppm mean measured concentration. Therefore, ADBAC is classified as highly toxic to sheepshead minnows. The NOEC was 0.68 ppm.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 10-09-92.

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Page ___ is not included in this copy.

Pages 18 through 20 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

RIFICI ADBAC QUAT SHEEPSHEAD MINNOW 10-09-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
2.8	20	20	100	9.536742E-05
1.8	20	20	100	9.536742E-05
1.1	20	20	100	9.536742E-05
.68	20	0	0	9.536742E-05
.42	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .68 AND 1.1 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .8648698

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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DATABASE ENTRY FORM
FOR ACUTE OR CHRONIC TOXICITY STUDIES

1. Chemical Alkyl Dimethyl Benzyl Ammonium chloride Shaughnessy 069105

2. Common Name Of Organism Tested Sheephead Minnow

3. Scientific Name Cyprinodon Variegatus

4. Age Of Organisms ~~NEG~~ Juveniles

5. Guideline No. 72-3

6. Type Of Dosing Method (Circle One) Or Study

1. Oral 2. Dietary 3. Reproduction 4. Static
(5.) Static Renewal 6. Flowthrough 7. Acute Contact
8. Other _____

7. % AI Of Test Substance Non Radiolabeled = 80.8

8. Study Duration (Hrs Or Days) 96h

9. Dose Type (Circle One) A. LD50 (B.) LC50 C. EC50 D. MATC

10. Toxicity Level A. mg/kg (B.) ppm C. mg/l D. µg/l E. ng/l
F. µg/bee G. Other

11. 95% C.L.s 0.68 – 1.1 LC50 = 0.86

12. Curve Slope N/A

13. NOEL 0.68

14. Study Date (YEAR) 1992

15. Study Review Date (YEAR) 1992

16. Category (Circle One) (CORE) SUPPLEMENTAL INVALID

17. MRID of Accession Number 424795-02

18. Laboratory Wildlife Int. Ltd.

19. Reviewer L. Ritci

20. For Reproductive Studies (avian or aquatic) Indicate Which Parameter Affected At What Toxicity Level.

Eggs Layed _____ % Cracked _____ % Viable _____
% Live Embryos _____ % Egghatch _____ 14D Survivors _____
Growth Effected at _____ Other Effects _____

22

DATA EVALUATION RECORD

1. **CHEMICAL:** Alkyl Dimethyl Benzyl Ammonium Chloride Shaughnessey No. 069105.

2. **TEST MATERIAL:** 1) ADBAC QUAT 80%; Lot No. 7293K, CP-161-1, 010 0879; 80.8% active ingredient; a yellow clear liquid. 2) ADBAC (^{14}C); Lot No. 920326; 12.62 µCi/ml; 97.99-98.74% radiochemical purity; a clear colorless liquid.

3. **STUDY TYPE:** 72-3. Mollusc 48-Hour Embryo-Larvae Study. Species Tested: Eastern oyster (*Crassostrea virginica*).

4. **CITATION:** Sved, D.W., J.P. Swigert, and G.J. Smith. 1992. A 48-Hour Static Acute Toxicity Test with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in Embryo Larvae of the Eastern Oyster (*Crassostrea virginica*). Project No. 350A-103. Prepared by Wildlife International Ltd., Easton, MD. Submitted by ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association, Washington, D.C. EPA MRID No. 424795-03.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
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KBN Engineering and
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Signature: Louis M Rifici
Date: 10/21/92

6. **APPROVED BY:**

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Signature: P. Kosalwat
Date: 10/21/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: Henry Craven
Date: 8/12/93

7. **CONCLUSIONS:** This study is not scientifically sound. Control mortality (47.8%) was unacceptably high. Based on normalized embryo-larvae mortality, the 48-hour LC_{50} was 55.0 ppb mean measured concentration. Based on abnormal development, the 48-hour EC_{50} was 49.1 ppb mean measured concentration. Therefore, ADBAC QUAT is classified as very highly toxic to eastern oysters. The NOEC was 25.0 ppb.

8. **RECOMMENDATIONS:** N/A.

UPGRADED SEE ATTACHED 11-1-95

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Eastern oyster (*Crassostrea virginica*) embryos were obtained from the Horn Point Environmental Laboratory (HPEL), Cambridge, MD. Four females and three male adult oysters were stripped of gametes. Egg and sperm suspensions were filtered and then mixed. After two hours, the suspension was examined to evaluate fertilization success and determine embryo density.

B. Test System: The test chambers were 250-ml glass beakers containing 200 ml of solution. They were held in a temperature-controlled water bath designed to maintain 25 ±2°C. The laboratory environment was maintained on a 16-hour light photoperiod with a light intensity of 240 lux. Thirty-minute dawn and dusk simulation periods were used.

A dispensing stock solution was prepared using an aliquot of the ^{14}C-ADBAC solution and nonradiolabeled test material diluted to a concentration of 0.18 mg/ml with reverse-osmosis water. The calculated specific activity of the stock was 0.19 mCi/mmol. An appropriate volume of the dispensing stock was added directly into the test chambers containing 200 ml of aerated dilution water and stirred for 2 minutes. Test concentrations were adjusted for the percent purity of the nonradiolabeled test material. The chambers were conditioned with test solution for 30 hours prior to test initiation.

The dilution water was filtered (2 µm) saltwater collected from the Choptank River at HPEL. The salinity at test initiation was 15 parts per thousand (ppt).

C. Dosage: Forty-eight-hour acute static toxicity test. Five nominal concentrations (18.1, 30.2, 50.4, 84.0, and 140 ppb) and a dilution water control were chosen for the definitive test.

D. Design: An appropriate volume of embryo suspension was added to each test chamber to achieve a density of 20-30 embryos/ml. The control group consisted of four replicate test chambers and each treatment group had

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three replicate chambers. Three surrogate test chambers were used to determine the initial embryo density and control mortality. Mean initial density was 228 embryos/10 ml. The test replicates were sampled 48 hours after test initiation to determine mortalities and abnormal development.

The dissolved oxygen concentration (DO) and pH were measured in each replicate at the beginning and end of the test. The temperature of one of the control chambers was monitored continuously. The temperature of each replicate vessel was measured daily. The salinity of the dilution water was determined at test initiation.

Water samples from each replicate were collected daily. The concentration of ADBAC QUAT (as mg ADBAC QUAT equivalents/l or ppm) was determined using liquid scintillation counting.

E. **Statistics:** Survival data for each treatment group were normalized for control mortality (47.8%). An LC_{50} value and 95% confidence interval was determined using the moving average method. An EC_{50} value was calculated (nonlinear interpolation and binomial probability) based on the number of dead larvae plus the number of larvae with incompletely developed shells.

12. **REPORTED RESULTS:** Five additional concentrations (233, 389, 658, 1080, and 1800 ppb) were tested but the biological results were not presented. Mean measured concentrations were 25.0, 40.8, 58.6, 89.7, and 145 ppb (Table 1, attached).

The mean number of larvae surviving the test period was 52.2% (119 embryos/10 ml) and was considered normal (Table 2, attached). The 48-hour LC_{50} was 55.2 ppb with a 95% confidence interval of 52.1-58.5 ppb. The 48-hour EC_{50} for normal oyster embryo development was 47.6 ppb (95% C.I. = 40.8-58.6 ppb). The no-observed-effect concentration (NOEC) for mortality and abnormal effects was 25.0 ppb.

During the test, the DO was >60% of saturation (6.2-7.2 mg/l). The pH values ranged from 7.6 to 7.8. The temperature was 24.5-25.5°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made other than those stated above.

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Good Laboratory Practice Compliance and Quality Assurance Statements were included in the report indicating compliance to with EPA Good Laboratory Practice Standards. Characterization of the test substance was the responsibility of the sponsor. The dates and types of quality assurance audits were also included in the report.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with the SEP and ASTM (1989), except for the following deviations:

Control mortality was 47.8% and considered normal for this source of oysters by the authors. The SEP and the ASTM states that no more than 30% mortality in the control oysters is acceptable.

The use of physically stripped gametes is not recommended by the ASTM. Spawning of conditioned oysters should be initiated by thermal stimulation.

The SEP states that the test should be initiated with embryos which are one hour post-fertilization. The embryos used in this test were approximately three hours old at test initiation.

B. Statistical Analysis: The reviewer used EPA's Toxanal program to calculate the 48-hour LC_{50} and EC_{50} values and obtained results similar to the authors' (see attached printouts).

C. Discussion/Results: This study is not scientifically sound. Control mortality (47.8%) was unacceptably high. The source of oysters used for testing or the methods used to obtain the gametes may not be suitable and must be evaluated by the laboratory. Based on normalized embryo-larvae mortality, the 48-hour LC_{50} was 55.0 ppb mean measured concentration. Based on abnormal development, the 48-hour EC_{50} was 49.1 ppb mean measured concentration. Therefore, ADBAC QUAT is classified as very highly toxic to eastern oysters. The NOEC was 25.0 ppb.

D. Adequacy of the Study:

(1) Classification: Invalid.

(2) Rationale: Control mortality (47.8%) was unacceptably high.

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(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 10-09-92.

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Page ___ is not included in this copy.

Pages 28 through 30 are not included in this copy.

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_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

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RIFICI ADBAC EASTERN OYSTER 10-09-92 ABNORMAL DEVELOPMENT

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
145	100	95	95	0
89.7	100	96	96	0
58.6	100	85	85	0
40.8	100	23	23	0
25	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 47.57255

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.0115627	49.14497	45.87694	52.40168

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	2.227689	36.10867	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 6.011119
95 PERCENT CONFIDENCE LIMITS =-2.960744 AND 14.98298

LC50 = 49.76219
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 30.59315
95 PERCENT CONFIDENCE LIMITS = 0 AND 54.87903

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RITICI ADBAC QUAT EASTERN OYSTER 10-09-92 Normalized Mortality

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
145	100	94	94	9.536742E-05
89.7	100	94	94	9.536742E-05
58.6	100	70	70	9.536742E-05
40.8	100	15	15	2.069473
25	100	0	0	9.536742E-05

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 51.67882

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.195013E-02	55.02275	51.52547 - 58.63574	

58.63574

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	1.051309	17.81205	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 5.816296
95 PERCENT CONFIDENCE LIMITS =-.1473475 AND 11.77994

LC50 = 55.28914
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 33.44166
95 PERCENT CONFIDENCE LIMITS = 0 AND 50.84433

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DATABASE ENTRY FORM
FOR ACUTE OR CHRONIC TOXICITY STUDIES

1. Chemical Alkyl Dimethyl Dibenzyl ammonium chloride Shaughnessy 069105

2. Common Name Of Organism Tested eastern oyster

3. Scientific Name ~~Crassostr~~ Crassostrea virginica

4. Age Of Organisms < 3 hours post-fertilization

5. Guideline No. 72-3

6. Type Of Dosing Method (Circle One) Or Study

1. Oral 2. Dietary 3. Reproduction (4) Static
5. Static Renewal 6. Flowthrough 7. Acute Contact
8. Other _____

7. % AI Of Test Substance non Radiolabeled 80.8

8. Study Duration (Hrs Or Days) 48-h

9. Dose Type (Circle One) A. LD50 (B.) LC50 (C.) EC50 D. MATC

10. Toxicity Level A. mg/kg ~~B.~~ (b) ppm C. mg/l D. µg/l E. ng/l
F. µg/bee G. Other

11. 95% C.L.s LC_{50} = 55.2 (52.1-58.5) EC_{50} = 47.6 (40.8-58.6)

12. Curve Slope N/A

13. NOEL 25.0

14. Study Date (YEAR) 92

15. Study Review Date (YEAR) 92-oct-09

16. Category (Circle One) CORE SUPPLEMENTAL (INVALID)

17. MRID of Accession Number 424795-03

18. Laboratory Wildlife Int. Ltd.

19. Reviewer LRIFICI

20. For Reproductive Studies (avian or aquatic) Indicate Which Parameter Affected At What Toxicity Level.

Eggs Layed _____ % Cracked _____ % Viable _____
% Live Embryos _____ % Egghatch _____ 14D Survivors _____,
Growth Effected at _____ Other Effects _____

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